Amendments to the Specification

Please replace the paragraph spanning pages 71 and 72 of Example 23 with the following paragraph:

The FimH adhesive protein on type 1 fimbriae is antigenically and structurally conserved (Abraham et al., 1988, Nature 336:682-684) and the amino acid sequence of the protein in all *S. enterica* serotypes for which sequence information exists are 98 to 99 percent identical (based on GenBank analysis). Therefore, the induction of a strong immune response, especially a mucosal immune response, will likely contribute significantly to the induction of cross-protective immunity. We (Lockman and Curtiss, 1990, Infect. Immun. 58:137-143; Lockman and Curtiss, 1992, Infect. Immun. 60:491-496; Lockman and Curtiss, 1992, Mol. Microbiol. 6:933-945) had previously found that bacterial cells expressing type 1 fimbriae were unaltered in virulence and colonizing ability but were more rapidly cleared from blood than mutants unable to synthesize type 1 fimbriae. Such cells are more susceptible to phagocytosis (Ofek and Sharon, 1988, Infect. Immun. 56:539-547) and may also be more rapidly cleared from lymphoid tissues. These attributes might be intensified with a vaccine strain genetically altered to over express either type 1 fimbriae or just the FimH adhesive protein, which in either case could lead to hyper attenuation of the vaccine strain to reduce its immunogenicity. We have therefore devised a means to construct a live recombinant attenuated *Salmonella* vaccine that will give a delayed over expression of the *S. typhimurium* FimH protein after the vaccine strain has colonized lymphoid tissues. We have Asd^+ vectors pYA3337 with the low copy number pSC101 *ori*, pYA3332 with the moderately low p15A *ori*, pYA3342 with the moderate to high pBR *ori* and pYA3341 with the high copy number pUC *ori*. All of these Asd^+ vectors that are diagramed in Figure 41 have the P_{trc} promoter to drive expression of genes cloned into the multiple cloning site whose sequence (that is the same in all four vectors) is presented in Figure 42. Transcription from P_{trc} promoter is repressed (prevented) if the LacI repressor protein is present in the cytoplasm of the bacterial cell. To achieve this, we have constructed as diagramed in Figure 43 the insertion-deletion mutation Δ*ilvG3*::TT *araC* P_{BAD} *lacI* TT and a suicide vector for its introduction into the chromosome of vaccine strains. When strains with this insertion-deletion mutation are grown with arabinose in the medium, LacI protein is synthesized. After immunization, LacI protein

decreases in concentration as a consequence of cell division and the degree of repression of a P_{trc} promoter would gradually decrease with an eventual high-level constituitive expression of any gene sequence controlled by P_{trc}. Further delay in de-repression of genes controlled by P_{trc} on Asd^+ vectors can be achieved, as describe in Example 17, by introducing into the vaccine strain the Δ*araBAD23* and Δ*araE25* deletion mutations using the suicide vectors diagramed in Figure 31. Figure 44 provides the nucleotide and amino acid sequences of the *S. typhimurium fimH* gene and FimH protein. The strategy, using PCR and the listed oligonucleotide probes to clone either the entire *fimH* gene or a sequence specifying its first 100 amino acids into any of the Asd^+ vectors diagramed in Figure 41 using the multiple cloning site diagramed in Figure 42, is diagramed in Figure 45. It is known that the first 100 amino acids of the FimH protein specify the adhesive properties of type 1 fimbriae (Thankavel et al., 1997, J. Clin. Invest. 100:1123-1126) and that immune responses to this 100 amino acid sequence block adherence of type 1 expressing bacteria to host cells possessing the receptor for type 1 fimbriae [[()]]. Construction of simple attenuated vaccine strains with either of the two *fimH* inserts into anyone of the four Asd^+ vectors introduced into strains with the Δ*ilvG3*::TT *araC* P_{BAD} *lacI* TT and with and without the Δ*araBAD23* and Δ*araE25* mutations will lead to comparative studies on the stability, colonizing ability and immunogenicity of each construct. A construction with the best attributes and inducing high mucosal and systemic antibody titers against FimH that block type 1 fimbriae-mediated adherence will be the basis for further modification and enhancement of a vaccine with other insertion-deletion and deletion mutations demonstrated to maximize induction of cross-protective immunity against enteric bacterial pathogens.